REVIEW OF X-RAY EFFECTS ON NEMATODES OF ANIMALS WITH NOTES ON EFFECTS OF IRRADIATION RATE ON *Heterakis gallinarum* (NEMATODA)

by

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INTRODUCTION

In this era of atomic energy the study of ionizing radiation and its effects on living organisms has steadily increased. Much of this work has been done in the field of human medicine, but a major contribution has been made by British workers in the field of veterinary parasitology. These workers developed "living antigens" by attenuation of nematode larvae with ionizing radiation. Since that discovery, much work has been done in an attempt to develop "living antigens" from other kinds of nematode larvae. Other practical uses of X-irradiation attempted in the field of parasitology have ranged from treatment of pork infected with Trichinella larvae to the direct radiation of laboratory animals in an attempt to affect internal parasites.

Experiments have been carried out by other workers to gain basic knowledge of radiation effects with no immediate regard for practical application of their results. In most of these experiments the radiation used has been applied at a single rate. The present work preliminarly investigated the effects of different rates of X-irradiation on Heterakis gallinarum (Shrank, 1788) Madsen 1949.

LITERATURE REVIEW

Ascarids

As early as 1904 Perthes reported that the earlier stages of Ascaris suis eggs were more sensitive to X rays than later stages. He also reported finding irregular cell masses developing in place of normal gastrulae following X-irradiation. The development of Parascaris equorum eggs was also slowed by X-irradiation according to Perthes (ibid).

Seide (1925) also reported the retardation in rate of development of Ascaris eggs when subjected to X-irradiation. According to Holthusen (1921), Ascaris eggs were very resistant to radiation between fertilization and first cleavage and most sensitive at first cleavage.

Embryonated eggs of Ascaris suum appear to be more sensitive to radiation than non-embryonated eggs according to Villela et al. (1958). They also reported that eggs subjected to a dose of 100,000 roentgens (r) failed to yield larvae in the lungs of guinea pigs.

Other studies concerning radiation of Ascaris lumbricoides and other ascarids at various stages of development were carried out by Shikhobalova et al. (1958). They irradiated the eggs of Ascaris lumbricoides, Ascaris suum, and Ascaridia galli at various stages of development, ranging from one blastomere to infective larvae, respectively. These eggs were most sensitive at the blastula, morula and early gastrula stages, as judged by the number of infective larvae and their reduced ability to migrate within the host. This migration was more affected when Ascaris suum and A. lumbricoides eggs were irradiated at the one blastomere stage than after embryogenesis was completed. Post embryonic development was affected by irradiation of unsegmented eggs and as a result the percentage of females was increased. Shikhobalova et al. (1958) also maintained that the effects of irradiation with X rays on Ascaris lumbricoides and Ascaris suum eggs before cleavage depended upon the irradiation dose. One hundred per cent to 60 per cent of larvae developed to the infective stage after irradiation with 2,000 to 15,000r, 5-26 per cent developed after 30,000r, and all eggs were killed by 40,000r. The development of larvae was generally

retarded (evident at 7,000r) and those that developed were less infective.

Shikhobalova et al. (1961) indicated considerable variation in the X-ray sensitivity of eggs from various species of Ascaris and Ascaridia. They suggest a comparative study of eggs obtained from feces of the hosts with varied ascarid populations would be of great interest.

Cook (1939) investigated the effects of low temperatures on the development of post-irradiated Ascaris eggs. He reported that one-cell eggs, when allowed to develop at 25° C immediately after exposure to a dose of 5,000r, showed only 1-2 per cent normal embryos, whereas eggs receiving the same dose and placed at 5°C for eight weeks developed approximately 45 per cent normal embryos when returned to 25°C. This would indicate a significant influence of low temperature upon recovery from irradiation effects. Although it is quite evident from the results of these experiments that recovery did occur, the delay in cleavage caused by a dose of 5,000r was the same throughout the experiment and showed no evidence of being influenced by low temperatures.

Babero (1952) found that exposure to 20,000 and 40,000r definitely retarded the development of Ascaridia galli eggs and induced greater morphological abnormalities in the larvae which subsequently developed than in those which developed from eggs exposed to 5,000 and 10,000r. There were no differences in the sizes of the larvae of the same age.

Ruff et al. (personal communication) repeating some of the work of Babero found that increased dosages (greater than 5,000r) of X ray given Ascaridia galli eggs before cleavage retarded development and increased mortality of the eggs. A peak of increased infectivity occurred among worms recovered from birds fed eggs subjected to 5,000r

prior to segmentation. Decreased infectivity was noted in birds fed larvated eggs irradiated at higher dosage levels (20,000-80,000r). Irradiation of eggs after larvation at levels of 5,000r - 20,000r significantly stimulated growth rate while irradiation of eggs before segmentation had no great influence upon the growth rates of worms developing from them. Higher irradiation dosages (40,000-80,000r) significantly decreased the growth rate of worms developing from both larvated and non-larvated eggs. The sex of mature worms recovered from larvated eggs exposed to X-irradiation were all female. Irradiation of non-larvated eggs did not condition sex of the worms.

Trichinella spiralis

Tyzzer and Honeiji (1916) subjected Trichinella spiralis in vivo (host intestine) to short wavelength X rays. Dosages sufficient to kill the mice did not prevent the development of the parasites.

Semrad (1937) noticed considerable variation in the susceptibility of Trichinella larvae to X rays since certain dosages injured some parasites, but failed to injure others. Encysted Trichinella larvae in vitro that have been exposed to 1,200r or more may continue development in the intestine of suitable hosts, but they do not produce young. Even at an exposure of 400r the resulting infection was attenuated as indicated by the failure of the nematodes to reproduce.

Immature forms of Trichinella spiralis were X-irradiated with varying dosages of roentgens Evans et al. (1941). It was found that at proper dosages, the radiation permitted the organism to grow to maturity, to undergo copulation, and to begin the development of the

embryo, but the radiation killed the embryos before their development was completed. They believed that it should be possible to ascertain whether an intestinal infection of Trichinella spiralis, without the subsequent muscle invasion, can produce resistance to infections of Trichinella. Levin and Evans (1942) also found it was possible to treat Trichinella spiralis larvae with roentgen radiation before infecting rats, so that the larvae grow to maturity in the intestine, but do not produce offspring. They found this intestinal infection alone to cause a strong host resistance to a second infection with Trichinella larvae. They theorized that the origin of a mechanism of host resistance to Trichinella is located in the intestine since an intestinal infection alone produces resistance.

Alicata (1951) reported that infective Trichinella larvae exposed to 10,000r of radiation failed to produce young when fed to susceptible hosts. This type of infection insured the host against subsequent muscle infection. Following radiation dosages of 15,000 to 20,000r a few larvae were able to reach maturity in the host's intestine. No larvae receiving 30,000r matured in the intestine. Even when the radiation dose was increased to 600,000r some live larvae were present in the intestinal tract 24 hours after infection - none were present after 48 hours. At higher radiation dosage (700,000r) larvae did not become established. These larvae were passed out in the feces during the first 24-hour-period after infection. Similar effects were noted in larvae irradiated at 0°C and 24°C. It is also interesting to note that the trichina larvae showed no recovery from irradiation effects even though they were refrigerated for one month following irradiation.

Evident morphological changes of recovered worms were recorded as shrinkage and degeneration of ovary, inability of eggs to cleave properly and develop into tadpole larvae, cuticular thickenings, and stunted growth.

Immunological study by Sadun et al. (1957) showed that rabbits receiving single innoculations by mouth of irradiated Trichinella spiralis larvae failed to produce antibodies in sufficient amounts to be detected by the complement-fixation test. Multiple feedings with irradiated larvae produced a positive serology of brief duration, at low titres. Larvae innoculated extra-intestinally stimulated the production of antibodies in relatively high titres. Accordingly, they warned that a re-evaluation of the significance of positive serology in diagnosing trichinosis might become necessary if commercially irradiated pork becomes available on the market.

Kim (1957) irradiated Trichinella spiralis larvae with varying dosages (1,750, 3,500, 5,250 and 7,000r, respectively,) in an attempt to eliminate one or more phases of the life cycle. These irradiated larvae were used in giving three stimulating infections. The degree of immunity produced was measured by adult worm counts and length measurements of female worms 7 days following a challenging infection with non-irradiated larvae and by larval counts 28 days following this challenging infection. The degree of immunity was compared with that produced in mice given the same number of stimulating infections with non-irradiated larvae. Using one or more of the three criteria of immunity it was shown that all of the groups given the stimulating infections had developed immunity. The results preclude that migrating

and encysting larvae are contributors to the total immunity. It is also clear that both the pre-adults are more important in this connection than formerly believed.

Larsh and Race (1958) found better immunity developed in mice when infected with natural Trichinella spiralis larvae rather than irradiated larvae. Those mice immunized with natural larvae showed a faster inflammatory reaction at the site of infection than the mice immunized with irradiated larvae. Larsh et al.(1959) later reported that mice given 5 stimulating infections with irradiated (7,000r) Trichinella spiralis larvae exhibited, after a challenging infection with non-irradiated larvae, about the same degree of immunity as those given the same number of previous infections with non-irradiated larvae, but the titre of serum antibody was much higher in the latter.

Alicata (1956) attempted to develop a strain of X-ray resistant Trichinella spiralis. The larval stages in six successive generations were irradiated with 5,000r, but the percentages of sterile females was not lower in the 6th generation than it was in the parent generation.

Gomberg and Gould (1953) found that about 1,000,000r of 200kv X rays are necessary to kill all trichina larvae in vitro, while Gould et al. (1953) reported a dose of 750,000r filtered X rays applied to trichina larvae in vitro killed nearly all one to two hours after irradiation.

Shikhobalova et al. (1958) discovered that 8,000 to 15,000r given Trichinella spiralis larvae encysted in meat stopped the development to maturity of both male and females. Shikhobalova (1958) X-irradiated pig muscle infected with Trichinella larvae with 1,000 to 3,000r.

These encysted irradiated larvae, when fed to mice, were eliminated within 2 days. The males were found to be less resistant to irradiation than the females. Shikhobalova et al. (1962) again reported that male Trichinella spiralis were more susceptible to X-irradiation than females. Complete sterilization of all worms was effective with doses between 7,000 and 9,000r.

Studying the X-ray sensitivity of eggs from Trichinella Shikhobalova and Paruzhinskaya (1961) noted the irradiation had more effect on the infective capability and post-embryonal development of the parasite when irradiation was applied to eggs containing infective larvae than when the blastomere stage was irradiated. Irradiation of completely embryonated eggs with doses of 20,000 to 40,000r destroyed the capacity for post-embryonic development. The same doses applied to the zygotic stage showed no inhibitory effect.

In order to determine the dependability of irradiation of pork for destruction of Trichinella larvae and the dose required Alicata (1948-1950) subjected slices of trichonosed pork and rat diaphragm to a radiation dose of 10,000r. The adult worms developing in rats fed this irradiated meat were sterile. At dosages of 30,000r several growing larvae were found in rats killed 24 and 48 hours after infection. Irradiation of 700,000r destroyed the larvae. Degeneration of the ovaries, interference with embryogensis, morphological changes and stunting of growth were effects reported. A dose of 12,000 to 14,000r was needed to abolish the reproductive capacity of Trichinella spiralis larvae (Zaiman et al., 1961).

Scardino and Zaiman (1962) noted a marked eosinophilia in rats infected with non-irradiated Trichinella spiralis larvae. No eosinophilia was demonstrated in similar rats each of which was infected with the same number of larvae sterilized by means of roentgen radiation. Zaiman et al. (1963) later reported eosinophilia did develop in rats infected with Trichinella larvae exposed to 14,000r. In another similar experiment, larvae subjected to 12,000r and then fed to mice, produced eosinophilia in the mice.

Dictyocaulus viviparous

Considerable X-irradiation work has been done with Dictyocaulus viviparous in preparing a "living antigen" vaccine. Jarrett et al. (1958) prepared a vaccine by attenuation of infective larvae with X-irradiation. A considerable degree of protection was conferred on vaccinates as judged by clinical and pathological findings, mortality, morbidity, fecal larvae counts and worm burdens. A much higher degree of protection was induced by giving two doses of irradiated Dictyocaulus viviparous larvae at about one month intervals according to Jarrett et al. (1959). Groups of 10 calves were used, both vaccinates and controls being challenged with 10,000 infective larvae. At autopsy the lungs of the vaccinated calves were normal while those of the controls contained a mean of 900 adult worms. The vaccine used was prepared by exposing the third stage larvae to 40,000r from an X-ray source.

Jarrett et al. (1961) later used two groups of calves, one group vaccinated twice with irradiated lungworm larvae, the other a control

group. These two groups were grazed on a pasture from which other calves contracted severe parasitic bronchitis. There was a marked difference in clinical signs, fecal output of larvae, worm burden, and growth rate of vaccinate and control calves, favoring the vaccinated calves.

Jarrett and Sharp (1963) reported a male-female ratio of 1:10 in the Dictyocaulus viviparous recovered from the lungs of a calf that had been fed irradiated larvae. The usual sex ratio is about 1:1.

Cornwell (1960) measured the antibody response of 32 calves vaccinated with a double dose of irradiated Dictyocaulus viviparous larvae vaccines by the complement-fixation test using a whole worm antigen. Titres resulting from the first dose were low while the second dose produced a variable rise in titre. Individual calves showed a wide variation in response and in three calves no antibodies were produced by either dose. Cornwell suggested that the antibody response may be due to irradiated larvae which succeed in establishing themselves in the lungs before being eliminated. Later Cornwell (1961) observed the serum antibody titre and respiratory rate in 26 calves vaccinated at a four week interval with two doses of irradiated Dictyocaulus viviparous larvae. Ten weeks after the first dose of vaccine the serum titre according to the complement fixation test showed wide variations (0-480) in individual calves. In 10 of 14 calves the respiratory rate rose to just over 40/min., 14-22 days after the first dose of vaccine, but the extent of the rise in individual calves was not proportional to the rise in serum titre. Where respiratory rates were already raised by an infectious, non-helminth associated respiratory disease (virus pneumonia), vaccination caused no further rise in respiratory rate or

other ill effect.

Cornwell and Berry (1960) warned if the resistance which follows vaccination (with irradiated Dictyocaulus viviparous larvae) is not sufficient to protect completely against natural infection, such an infection may produce carriers which are a danger to healthy stock.

Duwel (1963) organized laboratory and field experiments involving 1,612 calves 4-18 weeks old, of which 1,061 were dosed with X-irradiated infective Dictyocaulus viviparous larvae. The vaccine proved effective in preventing clinical dictyocauliasis with the following observations made: (1) all the calves in the herd must be housed for 3-4 weeks after vaccination, (2) calves younger than 8 weeks or those clinically affected with pneumonia and/or enteritis did not respond satisfactorily to vaccination, (3) vaccinates subsequently heavily infected with gastro-intestinal helminths may also develop clinical dictyocauliasis, (4) under pasture conditions in northwest Germany up to 67 per cent of calves vaccinated in 1963 produced larvae in their feces and (5) when natural infection was prevented 1.2 per cent of 580 vaccinated calves produced small numbers of larvae in their feces.

Two doses of irradiated 3rd stage lungworm larvae were given to susceptible calves (Edds et al., 1963) in control and field trials. Calves receiving the two doses were not affected by an average challenge exposure. Placement of vaccinated animals on infected pastures prior to the lungworm season was recommended to stimulate further immunity.

Engelbrecht (1961) treated 6-8 week old calves as follows. (1) Ten calves received 1,000 irradiated lungworm larvae, 40,000r. These calves were killed at intervals ranging from 42 hours to 29 days after

vaccination. No gross lesions attributable to the vaccination were noted. (2) Five calves received 1,000 non-irradiated larvae and were killed 42 hours, 90 hours, and 29 days, respectively, after being dosed. No larvae were found at 42 and 90 hours, but 71, 379, and 134 lungworms were found in those killed at 29 days. (3) Seven were given two doses of 1,000 irradiated larvae, 40,000r, within an interval of 30 days. They were killed at intervals ranging from 2-29 days after second dose. No gross lesions attributable to the vaccination were noted. (4) Three calves received two doses of 1,000 non-irradiated lungworm larvae 30 days between doses. One died 20 days after the 2nd dose. Severe pneumonia was noted in all three. (5) Five calves received two doses of 1,000 irradiated larvae, 40,000r, and were then challenged with 4,000 non-irradiated larvae. Very few gross lesions were noted; an average of 3.8 lungworms per calf were found in the lungs 31 days post challenge. (6) Three untreated controls were challenged and extensive pneumonic lesions were noted 31 days post challenge. An average of 636 lungworms per calf was found in the controls.

Jones and Nelson (1960) investigated cattle on 4,000 farms and reported failure of the lungworm vaccine in cattle on only 4 of the farms. Pneumonia was present in cattle on 2 of the farms. It was suggested that the pneumonia or sudden exposure to heavy infection interfered with immunity.

Additional studies showing the effectiveness of an irradiated lungworm used in the prevention of husk in cattle are reported by Nelson et al. (1961). It was estimated that parasitic bronchitis appeared in vaccinated cattle on only 0.35 per cent of the farms.

If vaccinated calves were exposed to massive challenges their immunity could be overcome.

After administering twenty-thousand irradiated lungworm larvae to a calf, 66 per cent of these larvae reached the lungs in 48 hours (Poynter et al., 1960). Guinea-pig experiments showed that X-radiation either affected the change between the fourth or fifth stages or affected the worms after their change into the fifth stage. They migrated to the lungs in the same manner as normal larvae.

Van Eck et al. (1960) reported that the vaccine prepared in the Netherlands consisted of a suspension of 1,000 - 1,500 X-irradiated 3rd stage larvae of Dictyocaulus viviparous in 20 ml of water. It was given by mouth and was repeated six weeks later. This vaccine was given to 708 calves on 75 infected farms. Even though the incidence of husk was generally low among unvaccinated cattle that year, the results were regarded as very satisfactory. The Dutch vaccine was used in Sweden by Olson (1962).

The optimal effective inactivation dose of X-ray exposure for 3rd stage larvae of Dictyocaulus filaria, intended as vaccine, was found to be between 40,000 and 60,000r (Jovamovic et al., 1961). The sex ratio of female to male in non-irradiated larvae was 1.62:1; in irradiated larvae 11.5:1. A high degree of immunity (81.8 per cent) against thread lungworm in sheep was obtained by double vaccination according to Sokolic et al. (1963). The antigen of this vaccine, infective larvae, was attenuated at a level of 40,000r X rays of roentgen origin. Immunity was indicated by noticeable differences in (1) average number of larvae/gram of feces/reproductive female recovered within

the entire post challenge period, (2) the number of parasites present, (3) the relative mortality, (4) the degree of clinical and pathmorphological changes, and (5) the degree of inhibition of parasitic development in general, but especially of oogenesis inhibition when comparing vaccinated, immune animals with non-vaccinated controls.

Hookworms

Bandyopadhyay et al. (1960) collected fecal samples containing suitable concentrations of hookworm eggs (Ancylostoma duodenale and Necator americanus). These samples were smeared on strips of sterile filter paper and exposed to 250, 500, 1,000 and 1,250r. Cultures were prepared from these paper strips. After 500r, formation of rhabditiform larvae occured 24 hours earlier than in the control. The attainment of the filariform stage in the 500r sample also preceded that of the rest of the test samples by 24-48 hours and the control by 72 hours. In general, X-radiation seems to favor an acceleration in the rate of larval development and early attainment of the infective filariform stage.

Dow et al. (1958 and 1959) immunized pups by vaccinating them with irradiated infective Uncinaria stenocephala larvae. Six dogs were vaccinated with 1,000 larvae subjected to 40,000r. One hundred twenty-eight days later, these animals and six controls of the same age were given 1,000 normal infective larvae. All were sacrificied 22 days later. The mean number of worms recovered from the control group was 530, and the corresponding figure for vaccinated group was 32. A high degree of resistance to U. stenochphala was reported when a double dose of irradiated infective larvae was given to pups. (Dow et al., 1961)

Miller (1964) first reported the use of X-irradiated Ancylostoma caninum larvae to produce immunity against challenging infections. He observed reduced fucundity of exposed larvae and lessened pathogenicity as dosage increased. Male larvae were reported to be more sensitive than females. Radiation doses of 40kr and above resulted in sterile females. A single subcutaneous vaccination of 3-month-old dogs with 1,000 attenuated larvae conferred highly significant resistance. A double subcutaneous vaccination with 40 kr-irradiated infective A. caninum larvae (Miller, 1965) conferred a high degree of resistance. Double subcutaneous vaccinations of 3- and 4-month-old pups were more effective than double oral vaccinations with the same preparation when resistance was measured by the establishment of adult hookworms resulting from an experimental challenge of normal larvae. In terms of resistance to the pathogenic effects of the challenge as demonstrated by hematologic and coprologic findings and by clinical observations, both methods of vaccination are equally effective in protecting the vaccinates when compared with the severely affected control dogs following challenge (Miller, 1965).

Other Species

Jarrett et al. (1960) administered X-irradiated (90,000r) Trichostrongylus colubriformes larvae to lambs and noticed an immune response capable of giving good protection. However, the X-ray dosage may need to be increased as the worms developed to the adult stage although they were mainly non-egg laying females.

In studying the effects of X rays on the infective larvae of Trichostrongylus axei (Ciordia and Bizzell, 1960) it was noticed that a dose of 90,000r failed to inhibit the motility of the infective larvae maintained in tap water at room temperatures for 28 days. A dose of about 5,000r apparently stimulated the infective power of the larvae without noticeable increase in their pathogenicity. Above 10,000r, fewer adult nematodes were present in the stomachs of infected rabbits. There was a gradual reduction in the proportion of male to female parasites recovered as the dose increased above 5,000r. No male worms were recovered from rabbits that received larvae exposed to 60,000r. No worms were recovered from rabbits given larvae exposed to 90,000r.

Thomas and Quastler (1949 and 1950) irradiated Rhabditis sp. larvae with doses of 10,000, 20,000 and 40,000r, respectively. The controls and those receiving 10,000r were normal when examined 18 days later. Those receiving 20,000r were examined 19 days later and many appeared with marked changes in the refractive index of the rhabditin granules of the intestine. The controls showed no such changes. The larvae receiving 40,000r were examined 10 days later and it was discovered no adult worms were present and only a few active larvae. The young females and males that were present were sterile as indicated by the vacuolate reproductive systems, also the irradiated worms showed looped or spiral-like intestines. Eggs appeared in assorted sizes, some with very thin shells.

Batches of infective larvae of Haemonchous contortus were treated with 10,000, 20,000, 40,000, 60,000 and 100,000r of X rays, respectively, (Jarrett, 1959). The larvae were then given orally to sheep to assess

the degree of inactivation achieved by irradiation and the immunity produced by such treated larvae. It was found that larvae subjected to 40,000 and 60,000r produced a good immunity to reinfection. Jarrett (1961) used two doses of vaccine (each containing 10,000 larvae treated with 40,000r) with lambs and reported sufficient immunity to withstand a challenge of 50,000 normal larvae.

Trichocephala muris eggs were irradiated (Shikhobalova and Paruzhinskaya, 1960) with a dose of 2,000-20,000r. The development of embryos in eggs exposed to 2,000-5,000r resembled that in untreated eggs, but at 10,000r development was delayed, at 40,000r only 1.7 per cent of embryos reached the invasive stage, and at 100,000r no embryos developed.

Shikhobalova and Paruzhinskaya (1962) irradiated Trichocephalus muris eggs at different stages of development with dosages of 5,000, 10,000 and 20,000r X rays respectively. Highest sensitivity to radiation was the 8-blastomere and morula stages. Few eggs given 5,000r at these stages developed into mature worms in mice. After 10,000r no infective larvae developed. Infective larvae that developed from eggs given 10,000 or 20,000r at the blastula stage did not reach maturity in mice. Syngamus eggs at the 4-8th blastomere stage were highly sensitive to a dose of 5,000r of X rays or more. Development was retarded and deaths occured at later embryonal or during larval development in experimentally infected chicks. An average of 4.9 pairs of Syngamus were found in each chick 21 days after an oral dose of 4,000r eggs that had developed after irradiation with 5,000r compared with 40.6 pairs in chicks given untreated eggs. Eggs containing infective larvae were also sensitive to radiation. Few worms reached maturity in chicks after exposure to 5,000r X rays,

but they largely resisted a dose of 2,000r X rays.

Variables other than X-ray dose which might influence the inactivation process of parasites were examined by Jennings et al. (1963). These variables were; (1) larval concentration during irradiation, (2) fecal contamination of the larval suspension and (3) rate of delivery of the X-ray dose. In an experiment with irradiated Nippostrongylus brasiliensis larvae the presence of fecal contamination, even up to 10 per cent dry matter in the solution, had no effect on the inactivation of the larvae. Larvae irradiated at a concentration of 1500 per ml were markedly more inactivated than those treated with the same X-ray dose at concentrations of 9,000 and 50,000 larvae per ml. There was no significant difference between the results obtained at the different dose rates.

MATERIALS AND METHODS

Collecting and Culturing of Nematode Eggs

Adult Heterakis gallinarum were collected from chicken ceca obtained from a poultry dressing house in Manhattan, Kansas. Ceca were flushed using a modified hydraulic method of Ackert and Nolf (1929) and worms were recovered by straining through a 40 mesh seive. The adult worms, which served as the source of eggs for the experiments, were macerated by means of a mortar and pestle and then treated with artificial digestive fluid (1.0 per cent pepsin and 0.5 per cent hydrochloric acid) for four to five minutes. The mixture was then filtered through an 80-mesh screen into Petri dishes (100 mm). Only a few mililiters of the mixture were poured into each dish. Tap water was added and after the eggs had settled to the bottom of the dish, the supernatent solution

was carefully decanted. The digestive fluid was removed by three to four additional washings with tap water. Twenty-five ml of 0.2 per cent formalin solution were added to each dish to inhibit mold and bacterial growth. The Petri dish cultures were then stored at 6°C until used.

X-irradiation of Eggs

A Picker 150kv X-ray unit at the Kansas State University Physics Department was used for all irradiations. The X-ray unit was operated at 90kv and 4ma in Experiment 1. Distance between X-ray source and eggs was approximately 14.3 cm for rate I (261.6 r/min.). The distance was doubled (28.6 cm) for rate II (134.4 r/min.) in Experiment 1. Rate II in Experiment 2 (larvated eggs) was obtained by lowering amperage to 2ma. A special wooden block was used to support the Petri dishes beneath the X-ray cone (Ostlind and Hansen, 1965). This block permitted determination of the radiation rate by removing the center plug and inserting a Victoreen r-meter thimble in its place. The thimble of the Victoreen meter was set so that its center would be in the same plane as the eggs in the Petri dish. Four calibration measurements, each for 2 seconds of exposure, were taken and the average radiation rate calculated. The average in Experiment 1 was 9.72 r/2 seconds for rate I. The average reading for rate II was 4.48 r/2 seconds. The same roentgen rates that were established in Experiment 1 were accepted for Experiment 2. Exposure time and other data are shown in Tables 1 through 4.

Non-larvated Heterakis gallinarum were irradiated in Experiment 1, whereas larvated eggs were irradiated in Experiment 2. In all cases the liquid depth in the dishes during irradiation was 2 mm. After recording the culture medium temperature the dish was placed on the radiation

Table 1. Exposure of Heterakis gallinarum eggs to X-radiation for Experiment 1, rate I.

Dose (r)	Time of exposure (min.)	Medium C°		Water depth in dish (mm)	Rate r/min.
		Before X ray	After X ray		
4,000	15.30	20.5	21.0	2	261.6
16,000	61.20	23.0	22.0	2	261.6
40,000	153.00	21.0	21.0	2	261.6
60,000	229.50	21.0	22.0	2	261.6
80,000	306.00	23.0	23.5	2	261.6
120,000	459.00	21.5	22.5	2	261.6

Table 2. Exposure of Heterakis gallinarum eggs to X-radiation for Experiment 1, rate II.

Dose (r)	Time of exposure (min.)	Medium C°		Water depth in dish (mm)	Rate r/min.
		Before X ray	After X ray		
4,000	29.75	22.0	20.0	2	134.4
16,000	119.00	23.0	21.0	2	134.4
40,000	297.50	23.0	21.5	2	134.4
60,000	446.25	21.5	21.5	2	134.4
80,000	595.00	22.5	22.0	2	134.4
120,000	892.50	26.0	23.0	2	134.4

Table 3. Exposure of Heterakis gallinarum larvated eggs to X-radiation for Experiment 2, rate I.

Dose (r)	Time of exposure* (min.)	Medium C°		Water depth in dish (mm)
		Before X ray	After X ray	
4,000	15.30	24.0	24.5	2
16,000	61.20	27.0	24.0	2
40,000	153.00	26.0	25.0	2
80,000	306.00	27.0	25.0	2

*Exposure time was calculated using same roentgen rate as in Experiment 1.

Table 4. Exposure of Heterakis gallinarum larvated eggs to X-radiation for Experiment 2, rate II.

Dose (r)	Time of exposure* (min.)	Medium C°		Water depth in dish (mm)
		Before X ray	After X ray	
4,000	29.75	24.0	24.0	2
16,000	119.00	25.5	25.0	2
40,000	297.50	27.0	25.0	2
80,000	595.00	26.0	15.0	2

*Exposure time was calculated using same roentgen rate as in Experiment 1.

block under the X-ray unit cone. The block supporting the Petri dish had been secured in a lead box to reduce stray radiation and standardized backscatter. The X-ray unit was turned on and after exposure time had elapsed, the unit was turned off and the Petri dish removed from beneath the cone. The temperature of the medium was taken immediately and the dish was returned to the incubator (30°C) where it remained until birds were to be infected, usually within 24-28 hours.

Development of Irradiated Eggs

The rate of development of the non-embryonated eggs in Experiment 1 was determined every 24 hours. The developmental stages of Ascaridia galli eggs, as described by Ackert (1931), were divided into four easily distinguishable stages. The developmental stages of Ascaridia galli eggs could be used because they are identical to the developing stages of Heterakis gallinarum. The four stages of development were: (1) Stage 1, fertile to and including the 4-cell stage; (2) Stage 2, 5-cell stage to and including last stage of the morula with large blastomeres; (3) Stage 3, morula with small blastomeres to and including last stage before becoming a tadpole larva; and (4) Stage 4, tadpole stage to infective larva.

Twenty-four hours after exposure each dish was removed from the incubator and the eggs examined microscopically with the aid of a Petri dish adapter (Ostlind and Hansen, 1964) to determine the stages of development. Each dish was examined systematically and the first 100 fertile eggs were counted and classified into one of the four previously described stages.

Preparation and Infection of Birds

Chickens used in the experiments were straight-run White Rocks obtained as day-old chicks from a commercial hatchery. They were raised on an 18 per cent protein, antibiotic free feed. The chicks were innoculated intranasally with New Castle Disease Vaccine* when they first arrived in the laboratory.

Birds were raised in electric brooders and transfered to battery cages when 14 days old. The birds were banded, weighed and placed in groups of approximately equal weights according to the method of Gardiner and Wehr (1950). All birds were infected at two weeks of age.

The method of infection for both experiments was the same and carried out in the following manner. Culture media were decanted from the Petri dishes and 5-15 ml of a 1.25 M sucrose solution were poured into the dish. Eggs were scraped from the Petri dish with the index finger and a rubber scraper. The egg suspension was then poured into a small bottle. A drop of the suspension was placed on a glass slide with a calibrated automatic micropipette and the number of eggs was counted under a compound microscope. The suspension was then diluted until the micropipette would deliver the desired number of eggs when filled to the calibration point.

Birds separated into designated groups were given $100^{+}_{-}10$ embryonated eggs in the sugar suspension *per os* by means of the calibrated pipette.

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*American Cyanimid, New York, New York

Laparotomy

The incidence of infectious enterohepatitis ("blackhead") was determined by laparotomy in the experimental birds 10-13 days after infection. Birds to be laparotomized were anesthetized with diethyl ether. Approximately 10 ml of ether were poured on absorbent cotton in a 5 ounce jar. The head of the bird to be anesthetized was placed in the jar without completely blocking the passage of air into the bottle. When proper anesthetization was obtained, the eyes remained closed and the leg muscles were fully relaxed. A one inch incission was made in the abdominal wall and both ceca were extracted with the help of a metal probe. Each cecum was examined for evidence of blackhead. A positive diagnosis was recorded only when a hard, caseous core was found in the cecum. The ceca were placed back in the coelom after examination and the incission was closed with wound clips.

Prepatent Period Determination

One bird from each group in Experiment 1 and two birds from each group in Experiment 2 were isolated on the evening of the 19th day after infection. The cecal feces from each bird was collected daily from the individual dropping pans beginning the morning of the 21st day after infection. A small amount of the feces was placed in a shell vial 1/3 full of $NaNo_3$ solution (sp. gr. 1.35). The fecal material was then agitated with a flattened end of a glass stirring rod before the contents were strained through a 40-mesh screen into a similar vial. The second vial was filled with additional $NaNo_3$ solution until a slight meniscus

was formed. A coverslip was carefully placed on this meniscus. Ten minutes later the coverslip was removed, placed on a glass slide, and systematically examined under a compound microscope for the presence of eggs.

Recovery of Worms

The birds were killed 30 days after infection. Each ceca was removed and put into a 2 ounce bottle half filled with saline. The bird's wing band was placed in one bottle with the left cecum, and a paper tag with the wing band number and the right cecum in another bottle. These bottles were refrigerated until processed.

After all the birds had been killed, each cecum in the bottles was processed individually in the following manner. The content of a single bottle were dumped into a 9 inch finger bowl and the bottle thoroughly rinsed with tap water. Tap water was added to the finger bowl to a depth of 3/4 inch. The cecum was cut open with a pair of scissors beginning at the proximal end. A pair of forceps was used to shake the fecal material free from the cecum. The cecum was then replaced in the bottle from which it was taken. The bottle was half filled with tap water, capped and shaken vigorously for 5-10 seconds. The contents were poured back into the finger bowl. The bottle was again rinsed into the finger bowl. The washed cecum was carefully removed from the bowl and discarded. The contents of the finger bowl were poured through a funnel into a quart jar. The funnel was then rinsed with enough water to fill the jar to the 600 ml mark, 55 ml of 10 per cent saline were added, which

resulted in a funal NaCl concentration of 0.85 per cent. The quart jar was placed in the refrigerator and its contents were allowed to soak overnight.

The following morning the quart jars were taken from the refrigerator, capped and shaken to break up the fecal material. The liquid was then decanted with a water faucet aspirator connected to a glass "J" tube leaving approximately 250 ml containing the sediment in the jar. The contents in the quart jar were shaken and poured into Pilsner glasses for further sedimentation. Then minutes later the supernatant was removed by the aspirator. The Pilsner glasses were shaken and their contents poured into separate 4 inch finger bowls. The entire contents of the bowl were examined systematically in a marked Petri dish under low power (.7 X) of a stereozoom binocular dissecting scope and the nematodes were picked up with a bent teasing needle. The worms were placed in small vials containing saline. Saline prevented the worms from bursting before they could be measured. The contents of each vial were emptied into a small Petri dish (60 mm) and the worms were sexed and counted. The whole worms were mounted on slides in saline and placed in vertical slide boxes under refrigeration until the worms could be measured.

Worm Measurements

The worms were taken from the refrigerator and placed on a bellow-type enlarger. The worm images projected on a ground glass plate were traced on tissue paper. The traced length of each worm was measured by a Dietzgen map measurer. Dividing the measured length by the proper reduction factor gave the actual length.

RESULTS AND DISCUSSION

Development of Eggs

Results of observations on the development of Heterakis gallinarum eggs are given in Tables 5 and 6. The percentage of eggs which failed to larvate was highest in the 120,000r, rate II group. The dosages showing the lowest larvation percentages at rate I were 120,000r and 60,000r. Highest larvation percentages were noted in the lower radiation dosages (4,000 and 16,000r) at both rates I and II. The middle radiation levels retarded the rate of egg development, but in some instances a lower radiation dose showed a slightly larger percentage of non-larvated eggs than a higher dose indicated.

Different degrees of larvation were noted (Table 7) in the eggs receiving the same dosages at two different rates, however, there was no trend for one rate to be consistently more detrimental than another rate. Fifty-eight per cent of the eggs receiving 60,000r (rate I) larvated at the end of 18 days while 73 per cent of the 60,000r (rate II) eggs larvated in the same incubation period. One the other hand 65 per cent of the eggs receiving 120,000r at rate I larvated while only 52 per cent of the eggs receiving the same dosage at rate II larvated. Table 7 also shows that irradiation decreases the rate of larvation when compared to the larvation rate of the control eggs. The higher irradiation dosages slow larvation rate more than the lower radiation levels. Different rates of the same dosage show varying results. However, one particular dose rate does not consistently appear more detrimental than the other. X-irradiation definitely slows the

Table 5. Progressive development of Heterakis gallinarum eggs in Experiment 1-irradiation rate I. Eggs (%) in each stage.

Dose (r)	Stage	Incubation (days)																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Control (0)	1	44	9	4	5	4	2	3	2	—	—	2	—	—	1				
	2	56	76	23	21	8	12	15	6	10	4	4	1	5	5				
	3	—	15	32	11	12	3	8	10	10	7	4	9	8	6				
	4	—	—	41	63	76	83	74	82	80	89	86	90	87	88				
4,000	1	88	12	12	3	3	2	—	2	2	2	—	1	1	1	—	1	1	2
	2	12	88	35	21	9	10	16	16	12	17	5	7	5	4	5	3	10	12
	3	—	—	49	27	8	5	4	11	16	7	20	12	13	13	18	16	17	3
	4	—	—	4	49	80	83	80	71	70	74	75	80	81	82	77	80	82	83
16,000	1	95	15	4	—	7	2	1	—	1	2	0	1	1	1	1	6	—	1
	2	5	84	38	8	3	9	13	13	7	4	6	16	4	3	5	4	2	6
	3	—	1	58	63	16	8	5	1	4	4	6	3	5	6	5	3	6	3
	4	—	—	—	29	74	81	81	86	88	86	88	80	90	90	89	87	92	90
40,000	1	100	62	27	25	7	10	4	—	4	—	3	1	—	2	2	3	6	1
	2	—	38	58	37	38	25	34	28	30	28	22	22	19	12	17	23	20	18
	3	—	—	15	22	18	12	15	8	11	5	14	12	15	16	19	12	10	12
	4	—	—	—	16	37	53	47	64	55	67	61	65	66	70	62	62	64	69
60,000	1	100	84	49	29	21	5	1	6	6	10	2	4	3	4	2	3	4	—
	2	—	16	49	44	32	37	34	32	36	28	30	23	25	30	19	31	27	32
	3	—	—	2	21	19	14	13	6	11	12	10	7	9	11	14	9	9	10
	4	—	—	—	6	28	44	52	53	47	50	58	66	63	55	65	57	60	58
80,000	1	100	94	65	37	13	16	22	8	5	1	6	3	3	1	—	—	2	1
	2	—	6	34	46	31	25	14	13	19	22	16	22	24	20	15	17	16	22
	3	—	—	1	17	47	36	16	7	7	2	6	5	1	9	5	7	11	7
	4	—	—	—	—	9	23	48	72	69	75	72	70	72	70	80	76	71	70
120,000	1	100	81	60	40	32	15	12	25	10	7	5	2	4	6	4	1	1	1
	2	—	19	40	39	37	32	31	18	26	26	23	14	27	20	23	22	19	22
	3	—	—	—	18	21	12	11	25	12	7	3	11	9	10	7	14	13	12
	4	—	—	—	3	10	41	46	32	52	60	69	73	60	64	66	63	67	65

Table 6. Progressive development of Heterakis gallinarum eggs in Experiment 1-irradiation rate II. Eggs (%) in each stage.

Dose (r)	Stage	Incubation (days)																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
4,000	1	73	22	4	5	2	1	3	--	2	5	--	--	1	2	2	--	--	1
	2	27	75	39	17	14	17	13	12	20	21	14	17	7	2	17	14	17	17
	3	--	3	57	14	14	4	1	4	2	4	4	8	13	19	11	9	9	5
	4	--	--	--	64	76	78	83	84	76	70	82	75	79	77	70	77	74	77
16,000	1	97	18	10	4	3	4	10	5	10	--	--	1	--	1	--	2	2	4
	2	3	82	56	22	15	11	8	15	10	12	7	15	10	13	14	12	13	8
	3	--	--	32	33	10	7	10	5	8	4	13	13	8	5	6	3	8	6
	4	--	--	2	30	72	78	72	75	72	84	80	71	82	81	80	83	77	82
40,000	1	100	40	21	4	2	5	1	2	1	4	--	1	1	--	2	--	1	--
	2	--	60	64	31	22	11	13	25	19	8	17	16	16	10	8	8	10	14
	3	--	--	15	55	23	19	12	4	4	16	8	3	3	12	10	17	10	9
	4	--	--	--	10	53	64	84	69	76	72	75	80	80	78	80	75	79	77
60,000	1	100	88	30	27	15	4	9	6	4	7	--	2	3	6	1	1	5	3
	2	--	12	68	56	44	27	21	22	27	19	17	17	19	17	16	17	19	19
	3	--	--	2	17	26	14	7	2	6	6	8	5	12	10	13	9	8	5
	4	--	--	--	--	15	55	63	70	63	68	75	76	66	67	70	73	68	73
80,000	1	100	81	49	28	14	10	13	13	6	4	2	3	4	4	1	--	--	--
	2	--	19	44	38	28	24	21	27	17	22	16	7	16	8	19	15	22	16
	3	--	--	7	31	30	22	11	8	8	2	6	16	10	6	4	6	13	12
	4	--	--	--	3	28	44	55	52	69	72	76	74	70	82	76	79	65	72
120,000	1	100	89	57	46	31	21	15	11	19	9	4	6	2	5	7	10	5	3
	2	--	11	43	45	36	33	29	23	25	22	27	24	32	33	26	22	23	30
	3	--	--	--	8	19	12	14	19	16	9	6	16	14	5	16	11	22	15
	4	--	--	--	1	14	34	42	47	40	58	63	54	52	57	51	57	50	52

Table 7. Percentages of larvation following irradiation of eggs at rates I & II in Experiment 1.

Dose (r)	Rate	Incubation (Days)			
		4	8	12	18
Control (0)		63	82	90	88
4,000	I	49	71	80	83
4,000	II	64	69	75	77
16,000	I	29	86	80	90
16,000	II	30	75	71	82
40,000	I	16	64	65	69
40,000	II	10	69	80	77
60,000	I	6	56	66	58
60,000	II	0	70	76	73
80,000	I	9	72	70	70
80,000	II	28	52	74	72
120,000	I	10	52	73	65
120,000	II	1	47	54	52

embryonation of the eggs, but it appeared that the rate at which the X rays are applied has no predictable effect upon the eggs.

Incidence of Blackhead and Patency

The incidence of blackhead in the experimental birds appears not to be related to the rate of X-irradiation dosage given to the eggs. The incidence of blackhead in Experiment 1 is relatively low in comparison with Experiment 2. The difference in the occurrences of blackhead in the two experiments could be related to different egg cultures used in the experiments. One egg culture could have possibly carried more Histomonas or a particular strain of Histomonas which was more pathogenic than the other. The incidence of blackhead in birds from the two experiments is shown in Tables 8 and 9.

In only one instance (16,000r, rate I) did irradiation extend patency beyond that of the controls in Experiment 1 (Table 10). In Experiment 2 (Table 11) there were three irradiation dosages (4,000r, rate II and 16,000r, rate I and rate II) that did not extend patency beyond that of the controls. Larvated, irradiated eggs used in Experiment 2 were more sensitive to X rays than the non-larvated eggs in Experiment 1 as shown by extended patency in Experiment 2. No eggs were found in the feces of any birds receiving eggs subjected to 40,000r at rate II, however, at necropsy immature females were recovered. Birds infected with eggs receiving 80,000r (rate II) likewise showed no eggs in their feces and no worms were recovered at necropsy.

During the patency studies, abnormal, vacuolated eggs were occasionally seen. It is possible these abnormal eggs were a direct result of radiation damage since they were not found in feces from

Table 8. Incidence of blackhead in Experiment 1.

Dose (r)	Rate	Birds (no.)	Birds with blackhead (no.)	Blackhead (%)
Control (0)		8	0	0.0
4,000	I	7	0	0.0
4,000	II	8	1	12.5
16,000	I	8	1	12.5
16,000	II	8	0	0.0
40,000	I	8	0	0.0
40,000	II	8	1	12.5
60,000	I	8	0	0.0
60,000	II	8	1	12.5
80,000	I	7	0	0.0
80,000	II	6	0	0.0
120,000	I	8	0	0.0
120,000	II	8	1	12.5

Table 9. Incidence of blackhead in Experiment 2.

Dose (r)	Rate	Birds (no.)	Birds with blackhead (no.)	Blackhead (%)
Control (0)		8	4	50.0
4,000	I	8	0	0.0
4,000	II	8	0	0.0
16,000	I	8	0	0.0
16,000	II	8	5	62.5
40,000	I	8	7	87.5
40,000	II	8	1	12.5
80,000	I	8	1	12.5
80,000	II	8	0	0.0

Table 10. Effects of irradiation (dosage and rate) on patency in Experiment 1.

Dose (r)	Rate	Wing band number	Patency (Days)								
			19	20	21	22	23	24	25	26	27
Control (0)		4942	-	-	-	-	+				
4,000	I	341	-	-	-	-	+				
4,000	II	1066	-	-	+						
16,000	I	319	-	-	-	-	-	-	+		
16,000	II	1043	-	-	-	-	+				
40,000	I	1037	-	-	-	+					
40,000	II	302	-	-	+						
60,000	I	1041	-	-	-	-	+				
60,000	II	1007	-	-	+						
80,000	I	314	-	-	-	-	+				
80,000	II	307	-	-	+						
120,000	I	1009	-	-	-	+					
120,000	II	1036	-	-	-	-	+				

Table 11. Effects of irradiation (dosage and rate) on patency in Experiment 2.

Dose (r)	Rate	Wing band number	Patency (Days)										
			19	20	21	22	23	24	25	26	27	28	29
Control (0)		2690	-	-	-	-	+						
		2612	-	-	-	-	+						
4,000	I	2636	-	-	-	-	-	-	-	-	+		
		2608	-	-	-	-	-	-	+				
4,000	II	2691	-	-	+								
		2617	-	-	-	-	-	-	-	-	-	-	+
16,000	I	3879	-	-	-	-	-	+					
		2687	-	-	-	+							
16,000	II	2616	-	-	-	+							
		2625	-	-	-	-	-	+					
40,000	I	2684	-	-	-	-	-	-	+				
40,000	II	3900	-	-	-	-	-	-	-	-	-	-	-
		2700	-	-	-	-	-	-	-	-	-	-	-
80,000	I	3881	-	-	-	-	-	-	+				
		2697*											
80,000	II	3885	-	-	-	-	-	-	-	-	-	-	-
		2626	-	-	-	-	-	-	-	-	-	-	-

*Bird 2697 was lost during the isolation period.

chickens infected with non-irradiated eggs. At necropsy it was discovered that many of the groups showing these peculiar eggs had only female worms present, and if males were present, they were far outnumbered by the females. It is possible that these eggs were being produced by unfertilized females. The absence of, or small numbers of males was the most evident reason for unfertilized females, but possible blockage of the female copulatory opening because of radiation damage is another explanation.

Recovery of Heterakis gallinarum

Data on the number and sex ration of Heterakis gallinarum recovered from birds at necropsy is given in Tables 12 and 13 for Experiments 1 and 2, respectively. The lower numbers of worms per bird in Experiment 2 again indicated that larvated eggs were much more susceptible to irradiation than the non-larvated eggs. The male:female ration recorded in Experiment 1 showed a definite decrease in the number of males, as compared with the number of females, as the X-ray dosage increased. A dose of between 40,000 and 60,000r was required in Experiment 1 to produce a marked change in the sex ratio. Similar observations were made by Ostlind, 1965 (personal communication) in experiments with Heterakis gallinarum. Radiation dosages of 16,000r and above were sufficient to cause a definite change in the sex ration in Experiment 2. Also, the decrease in the number of males recovered as the X-irradiation dosage was increased was evident in Experiment 2. Four of the groups in Experiment 2 (16,000r, rate II; 40,000r, rates I and II and 80,000r, rate I) produced only one male worm. It appeared that the rate at which a particular dosage was applied had no conclusive effect upon the

Table 12. Worms recovered at necropsy in Experiment 1.

Dose (r)	Rate	Birds (no.)	Avg./bird	Male:female
Control (0)		8	30.0	1:0.95
4,000	I	7	38.3	1:1.10
4,000	II	7	14.4	1:1.20
16,000	I	7	48.5	1:1.20
16,000	II	8	19.0	1:1.40
40,000	I	8	29.2	1:1.80
40,000	II	7	51.4	1:1.23
60,000	I	8	31.0	1:2.85
60,000	II	7	29.0	1:2.30
80,000	I	7	38.6	1:2.60
80,000	II	6	29.4	1:4.40
120,000	I	8	32.0	1:4.10
120,000	II	7	10.9	1:3.40

Table 13. Worms recovered at necropsy in Experiment 2.

Dose (r)	Rate	Birds (no.)	Avg./bird	Male:female
Control (0)		4	48.7	1:1.14
4,000	I	8	2.4	1:1.18
4,000	II	8	4.1	1:1.73
16,000	I	8	7.4	1:14.80
16,000	II	3	21.0	No males
40,000	I	1	2.0	No males
40,000	II	7	4.6	No males
80,000	I	7	0.9	1:6.00
80,000	II	8	0.0	No worms

average worm burdens. Some dosages at rate I show higher worm burdens than the same dosage at rate II, but other dosage levels showed the opposite effects. The worm burden variations between rate I and II of each dosage indicated no effect of rate change on worm burdens. Worm burdens, regardless of rate or dose, are lower from eggs exposed to irradiation in the larvated stage.

Worm Measurements

Data from Experiments 1 and 2 in Tables 14 and 15 show a size difference between irradiated and non-irradiated groups. In both experiments there was a trend toward an inverse relationship between the rising radiation dosages and the length of the worms. The females measured in Experiment 2 recovered from the high radiation groups were smaller than the females recovered from corresponding groups in Experiment 1 indicating a marked sensitivity of larvated eggs to irradiation. The rate of X-ray application did not appear to condition size of worms within a given roentgen dosage.

The results obtained from these experiments showed similar results for a particular dose at both rates used. The over-all X-ray effect apparently depended on the total roentgen dosage rather than the rate.

SUMMARY

A review of the literature showed that most X-irradiation studies have been done in an attempt to develop "living antigen" vaccines. British workers made a vaccine from X-irradiated Dictyocaulus viviparous larvae that has proved to be highly effective in prevention of subsequent

Table 14. Effect of irradiation on worm length in Experiment 1.

Dose (r)	Rate	Avg. length of males (mm)	Range (mm)	Avg. length of females (mm)	Range (mm)	Avg. both sexes (mm)
Control (0)		8.620	7.20-10.00	10.160	8.00-12.00	9.38
4,000	I	8.300	7.20-10.40	9.728	8.00-12.00	9.00
4,000	II	8.780	7.60-10.00	10.160	8.20-12.20	9.52
16,000	I	7.560	6.00- 9.40	8.646	6.00-10.20	8.10
16,000	II	6.908	4.00- 8.40	8.000	6.20- 9.80	7.56
40,000	I	7.260	3.80- 8.80	8.564	5.00-10.20	8.10
40,000	II	7.488	4.60- 9.00	8.324	3.80-10.20	7.94
60,000	I	7.220	4.40- 8.60	8.086	3.60-10.00	7.86
60,000	II	7.420	3.60- 8.80	8.820	5.80-10.40	8.34
80,000	I	7.440	5.00- 9.00	8.860	6.60-10.40	8.48
80,000	II	7.460	5.20- 8.60	7.945	6.00- 8.80	7.70
120,000	I	7.270	5.80- 8.40	7.720	4.00- 8.40	7.50
120,000	II	6.160	2.80- 7.63	7.630	3.60- 9.60	6.90

Table 15. Effect of irradiation on worm length in Experiment 2.

Dose (r)	Rate	Avg. length of males(mm)	Range (mm)	Avg. length of females(mm)	Range (mm)	Avg. both sexes(mm)
Control (0)		8.84	4.20-10.00	9.30	4.40-12.00	9.70
4,000	I	7.46	5.00- 8.20	10.11	8.40-12.00	8.62
4,000	II	7.56	6.00- 8.20	9.25	6.00-10.20	8.66
16,000	I	6.44	6.00- 8.20	8.77	4.20-10.20	8.50
16,000	II	6.50	One male	8.94	6.00-10.80	8.90
40,000	I	---	---	9.40	9.20- 9.60	---
40,000	II	---	---	6.60	4.00-10.00	---
80,000	I	---	---	5.68	3.40- 7.00	---
80,000	II	No worms recovered				

infections. Male Dictyocaulus larvae were found to be more sensitive to X-irradiation than the female larvae during the course of the experiments. Other workers experimenting with Trichinella spiralis have had less spectacular results, but varying degrees of immunity have been established against trichinosis in laboratory animals. Successful, preliminary investigations also have been reported using irradiated hookworm larvae as a vaccine against Ancylostoma caninum infections.

X-irradiation studies have been done with the eggs of three species of ascarids-Ascaris suum, Parascaris equorum and Ascaridia galli. The segmented eggs from the above species were found to be more sensitive to irradiation than the unsegmented eggs. Those eggs reaching complete larvation were the most sensitive. The larvation rate of the developing eggs was progressively slowed as the irradiation dosage increased. Eggs from those nematodes that were refrigerated after X-irradiation seemed to partially recover from the detrimental irradiation effects.

Limited X-irradiation studies have also been done with species from the genera Trichostrongylus, Rhabditis, Haemonchous, Trichocephala, Syngamus and Nippostrongylous.

X-irradiation of Heterakis gallinarum eggs had several effects on the eggs and the worms developing from irradiated eggs. Larvated eggs are more sensitive to X rays than non-larvated eggs as indicated by (1) smaller worm burdens, (2) abnormal sex ratios, (3) decreased lengths and (4) increased pre-patent periods. The number of worms recovered from birds infected with X-irradiated, larvated eggs was lower than the number taken from birds fed control eggs. X-irradiation reduced the per cent of eggs reaching the larvated stages in all cases. Those groups

receiving the highest dosages showed the greatest per cent reduction of larvation at the end of the incubation period. The rate at which the eggs larvated was also reduced by the higher dosages of irradiation.

X-irradiation did not appear to affect the incidence of blackhead disease but the experiments were inconclusive.

The application of a particular X-ray dosage at two different rates appeared to have no differential effect on the eggs or worms.

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REVIEW OF X-RAY EFFECTS ON NEMATODES OF ANIMALS WITH NOTES ON EFFECTS OF IRRADIATION RATE ON *Heterakis gallinarum* (NEMATODA)

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AN ABSTRACT OF A REPORT

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A review of the literature showed that most X-irradiation studies to date have been done in an attempt to develop "living antigen" vaccines. A useable vaccine against Dictyocaulus viviparous infections has been developed, but possible vaccines against other nematode infections are still being investigated.

Nematode eggs from species such as Ascaris suum and Ascaridia galli have been subjected to X-irradiation. Eggs from these species were found to be more sensitive in the segmented stage. The rate of development of unsegmented eggs was also slower after X-irradiation treatment.

Nematodes from the genera Trichostrongylus, Rhabditis, Haemonchous, Trichocephala, Syngamus and Nippostrongylus have also been irradiated. All of the above species showed various degrees of inhibition as the result of the irradiation.

The present study was conducted to determine the effects of X-irradiation on the embryogeny of Heterakis gallinarum eggs and the larvae and adults developing from them. The dosage rates of X-irradiation were 261.6 r/min. and 134.4 r/min. for rates I and II respectively.

Larvated and non-larvated eggs were subjected to X-ray dosages ranging from 4,000 to 120,000r. Radiation effects were determined by recording developmental rate of eggs, incidence of blackhead, length of prepatent period, worm burdens, sex ratios and worm lengths.

Results of these experiments showed that irradiation reduced the larvation rate of the nematode eggs. The percentage of eggs reaching the larval stage was also lowered as the roentgen dosage increased.

Larvated eggs were more sensitive to radiation than non-larvated eggs as indicated by smaller worm burdens, larger percentage of females

than males in recovered worms (indicating potential males are more sensitive than potential females), decreased worm lengths, and increased prepatent periods.

No conclusions concerning the relationship of X-radiation and the incidence of blackhead could be made.

Similar results were obtained when a particular X-ray dosage was applied at two different rates. Total roentgen dosage appeared to be more important than the varying rates at which it was applied.